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Research Article

Gel formulations containing Sumbawa horse milk with carbomer gel base

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ABSTRACT

Peptides from Sumbawa horse milk are active as antibacterial and antioxidant. Horse milk has a higher content of vitamin C than cow or goat's milk, and cream products that contain milk can overcome dry skin. The Gel is one of the topical **4** sage forms suitable for acne medications and oily skin types. This study aimed to determine the effect of variations in horse milk content formulated in gel preparations with carbomer 1% base as gelling agents on physical characteristics (organoleptic, viscosity, pH) and antibacterial activity in *Propionibacterium acnes*. In this study, gel preparations were made with six different formulas on horse milk content, namely FI (2.5%), FII (5%), FIII (10%), FIV (15%), FV (20%), and FVI (25%) with 1% carbomer base on each formula and well method were used for the antibacterial test. From the organoleptic test, it was found that in all of the gel formula had soft textured, thick white color, and distinctive aroma like horse milk. The viscosity test showed that was no significant difference, but the pH test showed a significant difference for each formula. In this study showed that all of the gel formulae did not have an inhibitory zone, which means that at each level there was no evidence of antibacterial activity in the *P. acnes*. So, it could be concluded that all gel formula had good gel characteristics but there was no inhibitory activity against the *P. acnes*.

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INTRODUCTION

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Sumbawa horse milk is useful as an antibacterial against *Staphylococcus epidermidis*, which is one of the bacteria that causes acne. Also, Sumbawa horse milk has a feature that is its resistance to contamination of decay microorganisms so that the milk is durable (Laili, Setyowati & Irvati, 2014; Riyadh, 2003). The hydrolyzed peptide from horse

milk using the *Bacillus thuringiensis* protease is active as an antibacterial and antioxidant. A peptide from fraction < 3 kDa has the potential as an antibacterial (Kusumaningtyas, Widiastuti, Kusumaningrum & Suhartono, 2016). In Sumbawa horse milk, a galactoferrin compound found that has great antibacterial activity. The antibacterial properties in Sumbawa horse milk **2** have a broad spectrum, and it turns out that Gram-positive

bacteria are more sensitive than Gram-negative bacteria. Sumbawa horse milk has the best antibacterial activity when tested against several test bacteria, with cow milk and other horse milk as comparative (Hermawati *et al.*, 2004). In general, milk from cows and goats has a low content of lactose, high in protein and casein, fat, vitamins except for vitamin C when compared to horse milk. Horse milk has a higher vitamin C content (Clayes, 2014).

Dry skin is a condition where the skin loses moisture in its intercellular system. The texture of the skin becomes rough and feels itchy. Cream products that contain milk can prevent moisture loss (Yagil, 2017). Gel is one of the topical dosage forms that are still in demand by consumers and the drug and cosmetics industries. Gel with optimum properties can improve therapeutic effectiveness and comfort of use. The optimum gel physical properties can be obtained by optimizing the gel formula by combining two or more different bases. The good gel form is used to treat acne because gel preparations with a polar solution are easier to clean and do not contain oil, which will aggravate acne (Sasanti, Wibowo, Fidrianny & Caroline, 2012).

In the preparation of gel dosage form, a gelling agent and additives are needed according to the needs to produce cosmetic products according to the characteristics, the gelling agent used in this formulation is carbomer which has coolant characteristic, easily washed with water, good dispersion on the skin, releases the drug well, and does not clog the pores of the skin which is suitable for acne treatment (Allen, 2002).

From the description above, this study was conducted to determine the physical-chemical characteristics (viscosity, dispersion, pH) and antibacterial activity for gelatin-based gel preparations containing horse milk which were tested for antibacterial activity so that they could be used for acne-prone and oily skin.

RESEARCH METHODS

Material

The materials used in this study was horse milk from Sumbawa, carbomer (Corel Pharma Chem.), triethanolamine (Petronas Chem), glycerin (Wilmar Nabati), nipagin (Chemco), and distilled water (Brataco). As for conducting bacterial activity tests, *Propionibacterium acne* bacteria were used.

Preparation of Horse Milk Gel Dosage Form

First of all, develop carbomer with aquadest, wait until all carbomers were wetted. Add triethanolamine (TEA) until the pH is neutral and the carbomer expands perfectly. The dissolved nipagin added to the glycerin stirred until homogeneous. When the carbomer has formed into a gel, add TEA and scour until expands. Add the nipagin and glycerin mixture to the carbomer mixture and then crush until homogeneous. Then add the Sumbawa horse milk, stirring until it formed a homogeneous gel mass.

Dosage Form Evaluation

Organoleptic test

Organoleptic observations were carried out by looking at textures, colours, and scents of gel dosage form.

pH test

The electrode from the pH meter is washed with aquadest and dried. pH meter was calibrated with a standard pH solution starting from pH 4, 7, and 9. 1 g of the gel was dissolved ad 10 ml of aquadest and stirred ad homogeneous. The pH of the gel was measured by inserting the electrode into the gel and then the results were recorded.

Viscosity test

A total of 25 grams of the gel was prepared in a beaker glass, then a 64-size spindle was installed and the rotor was carried at 30 rpm. After the viscometer shows a stable number, the results were recorded and then multiplied by a conversion factor.

Antibacterial Activity Test

MHA (Mueller Hinton Agar) media preparation

Weighed as much as 15 grams of MHA and put into Erlenmeyer. Added with 1000 ml of aquadest, then heated over the hotplate until boiling while stirring until homogeneous. Then the media was sterilized using the Erlenmeyer mouth section covered with cotton and paper tied with rubber bands, then put into an autoclave for 15 minutes at 121 °C.

Bacterial suspensions preparation

Test bacterial culture (*P. acnes*) in slanted Nutrient Agar (NA) was taken 1 ose and inoculated in 10 ml of pro-injection aquadest, homogenized with vortex, then it was checked for turbidity with MPA density meter with various absorbents 0.08 - 0.1 equivalent with Mc Farland 0.5 with a bacterial concentration of 1×10^8 CFU/mL.

Table 1. Gel formulation of Sumbawa's horse milk.

| Formulation Ingredients | Pharmacotechnical Function | Ratio (%) | 3 | | | | | |
|-------------------------|----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | | Negative Control | Formula I | Formula II | Formula III | Formula IV | Formula V | Formula VI |
| Horse Milk | Active Compound | 0 | 2.5 | 5 | 10 | 15 | 20 | 25 |
| Carbomer | Gelling Agent | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| TEA | Alkalizing Agent | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Glycerin | Humectant | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Nipagin | Preservative | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Distilled water | Solvent | Sqf ^a | Sqf ^a | Sqf ^a | Sqf ^a | Sqf ^a | Sqf ^a | Sqf ^a |

^a Sufficient quantity for 100%.**Table 2.** The results of pH and viscosity test of Sumbawa's horse milk gel formulation

| Test | FI | FII | FIII | FIV | FV | FVI |
|-----------------|-------|-------|-------|-------|-------|------|
| pH | 6.01 | 5.96 | 5.94 | 5.55 | 6.24 | 6.46 |
| Viscosity (Cps) | 12200 | 11800 | 11366 | 12700 | 11000 | 9066 |

Table 3. The results of antibacterial activity

| Sample | Inhibition zone diameter (mM) |
|------------------|-------------------------------|
| Positive control | 23.3 |
| Negative control | 0 |
| FI | 0 |
| FII | 0 |
| FIII | 0 |
| FIV | 0 |
| FV | 0 |
| FVI | 0 |

Antibacterial activity test

The suspension of *P. acnes* test bacteria in suspension was taken using 100 µl of micropipette inserted into a petri dish, then MHA media was poured into sterile petri dishes aseptically 20 ml each, homogenized by shaking to form number 8. The Petri dishes were carried out in LAF (Laminar Air Flow), then a hole (well) was made with a diameter of 6 mm using a sterile hole tool. Gel test solution of horse milk with 2.5%, 5%, and 10% levels, positive control using clindamycin tested was taken as much as 20 µl, then put into the well and left to diffuse, then incubated at 37°C for 24 hours. A clear area that shows the inhibition area around the well was measured starting from the edge of the well using a calliper.

Data Analysis

The gel that has been made then tested for physical, chemical, and antibacterial characteristics and then

performed statistical tests using One Way ANOVA and Tukey HSD.

RESULT AND DISCUSSION

Organoleptic Test

The organoleptic observations of horse milk gel dosage form consist of colour, odour, and texture can be seen (Fig. 1). From the six formulas that have been made, all of them had the same organoleptic, which was thick white, a distinctive smell of horse milk, and had a soft texture.

pH and Viscosity Test

Based on the data (Table 2), the gel viscosity test results have been obtained. From the viscosity test profile, there appeared a decrease in viscosity in formulas V and VI. The lowest viscosity found in formula VI which have the largest milk content of 20%.

To determine the effect of increasing levels of horse milk used in the formula, a statistical analysis was performed using the One Way ANOVA test to show that there was no significant difference in viscosity in the formula. So it could be concluded that the increase in horse milk levels does not affect the viscosity of the preparation.

The pH data (Table 2). showed that the pH is still appropriate with the pH of the Indonesian National Standard (SNI), which is in the range of 5.55-6.46. From the results, a statistical test using One-way ANOVA was carried out showing significant pH differences in each formula.

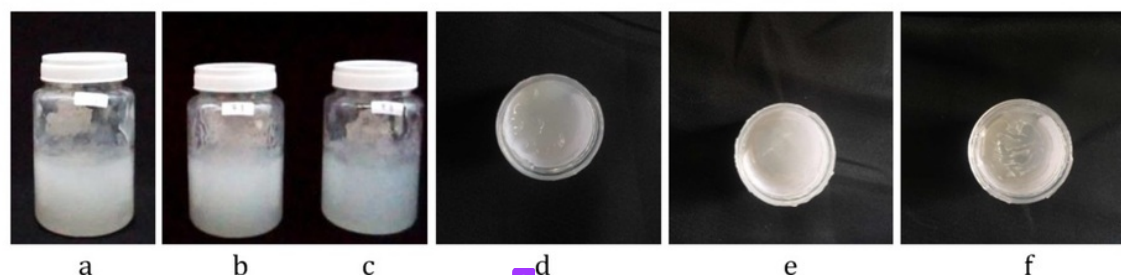


Fig 1. Organoleptics of Sumbawa's horse milk gel formulation. Formula I (a); Formula II (b); Formula III (c); Formula IV (d); Formula V (e); Formula VI (f).

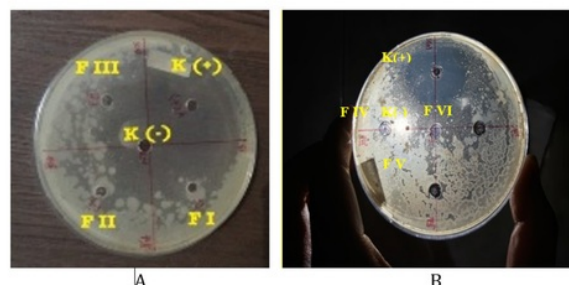


Fig 2. Inhibition zone diameter of FI, FII, and FIII (Left); FIV, FV, and FVI compare with positive and negative control (Right).

4 Antibacterial Activity Test

The results of the examination of the antibacterial activity test for horse milk gel dosage form could be seen in the table above (Table 3).

In testing the antibacterial activity against *P. acnes*, bacteria did not show any inhibition zones which were indicated by the absence of bright areas around the pit in each gel preparation level compared to the area of inhibition zone produced positive control (clindamycin) with inhibition zone diameter of 23.3 mm.

In several studies conducted by Laili et al (2014), showed that horse milk from Dompu District had antibacterial activity in *S. epidermidis* bacteria. The research conducted by Hermawati et al (2004), found a compound called galactoperrin, which has excellent antimicrobial activity. The antimicrobial properties of Sumbawa horse milk have a broad spectrum, and it turns out that Gram-positive bacteria were more sensitive than Gram-negative bacteria, horse milk used in this study came from Bima Regency.

The use of different bacteria can cause the absence of barriers in horse milk gel dosage form because bacteria have different properties and resistance to an antibacterial although the bacteria in one group are the same which is a Gram-positive bacterium. *S.*

epidermidis has a coagulase factor which causes peptidoglycan on the cell wall of the bacterium to easily clot, therefore *S. epidermidis* is more easily inhibit than *P. acnes*. Although it has no antibacterial activity against *P. acnes*, this gel can be developed as an antioxidant preparation and increases skin moisture.

CONCLUSION

Sumbawa horse milk can be formulated in the form of a gel with a carbomer 1% as a gelling agent with horse milk levels starting from 2% to 20%. All of the formula showed good gel characteristics, but there was no inhibitory activity against the *P. acne* bacteria.

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